RESEARCH NOTE

Neutralization of Primary HIV-1 Isolated from Individuals Residing in Rio de Janeiro

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Control of HIV-1 infection has been attempted to achieve by induction (Karzon et al. 1992 *Vaccine 10:* 1039-1052) or passive transfer of HIV-1 neutralizing antibodies (D Vittecoq et al. 1995 *Proc Natl Acad Sci USA 92:* 1195-1199, PWHI Parren et al. 1995 *AIDS 9:* F1-F6, MC Gaudin et al. 1995 *J Inf Dis 171:* 1203- 1209). Evaluation of the efficacy of anti-HIV/AIDS vaccine candidates has been based on analyses of the effective humoral immune response presented by neutralizing antibodies and of the effective cellular immune response presented by cytotoxic T lymphocytes (KB Cease & JA Berzofski 1994 *Annu Rev Immunol 12:* 923-89). The difficulty in inducing an effective immune response against HIV-1 has been shown to be caused by the extreme variability of this virus, used by this agent in order to escape immune control (JA Levy 1993 *Microbiol Rev 57:* 183-263). The initial optimism in relation to neutralizing antibodies (NAb) has thus decreased progressively in the last years, mainly after constatation that the satisfactory neutralization of laboratory HIV-1 isolates *in vitro* by patient sera cannot be presumed to indicate the capacity to neutralize the autologous HIV-1 isolate or the viral variants which appear during the progression of the immunodeficiency syndrome (DC Montefiori et al. 1991 *Virol 182:* 635-643). However, recent data indicate that potent broadly reactive, protective neutralizing antibodies do exist in patient sera and can be produced *in vitro* (JR Mascola et al. 1994 *J Inf Dis 169:* 48-54).

Exhaustive efforts aiming to identify HIV-1 variants in different geographical areas or defined groups of infected persons have been carried out. Although genotypic typing has identified different clades of HIV-1, serotyping efforts have not corresponded at the same level, and definition of main HIV-1 variants through neutralization studies has shown to be extremely difficult (S Osmanov 1995 personal communication). However, studies with monoclonal HIV-1 neutralizing antibodies have shown that some kind of "grouping" of HIV-1 variants according to their susceptibility to neutralization can be achieved (JP Moore 1994 *9éme Coll Cent Gardes* 151-155, DR Burton 1994 *9éme Coll Cent Gardes* 167-175, EM Fenyoe 1994 *9éme Coll Cent Gardes* 103-107, M Robert-Guroff et al. 1994 *J Virol 68:* 3459-3466, AJ Conley et al. 1994 *J Virol 68:* 6994-7000, JP Moore et al. 1995 *J Virol 69:* 101-109, E Jurkiewicz et al. 1995 *AIDS 9:* 91-93).

Several anti-HIV/AIDS vaccine candidates are being evaluated in diverse clinical trials. Brazil is one of the countries with some regions in the southeast of the country afflicted with a very high regional HIV-1 incidence, and the need for vaccines is urgent. In order to choose vaccine candidates to be applied in Brazil, basic studies not only of the viral variants circulating in Brazil but also of the immune response of infected individuals have to be carried out.

The present study presents results obtained analyzing the susceptibility to neutralizing antibodies of 27 primary HIV-1 isolates obtained from infected individuals residing in the city of Rio de Janeiro (Table). Autologous neutralization (defined as the capacity of antibodies in sera or plasma in neutralizing the HIV-1 isolate obtained at the same date from the same individual), heterologous neutralization (susceptibility to neutralization by NAb in plasma from other HIV-1 individuals), susceptibility to neutralization by control NAb (sera or pools of plasma known to neutralize primary HIV-1 isolates) and the capacity to neutralize a reference HIV-1 isolate (laboratory strain HIV-1 MN)

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TABLE

Summary of the results obtained in neutralization assays of 10-50 infective units of Brazilian primary isolates or of the reference HIV-1 MN isolate by antisera or plasma diluted 1:10

Neutralization	Extent of N[a]	# neutralized/ / # tested	%
Autologous N	50%	11/16	69
	80%	7/16	44
Heterologous N	50%	53/75	71
N by plasma pools	50%	12/12	100
		17/20	85
N by anti-HIV-1 MN	50%	6/12	50
N of HIV-1 MN	50%	25/28	89

[a]: neutralization

were determined. Traditional neutralization assays using a mixture of phytohemagglutinin (PHA) activated mononuclear cells obtained from HIV-1 seronegative blood donors were carried out (J Albert et al. 1993 *AIDS Res Human Retrovir 9:* 501-506).

Results indicated a fairly high percentage of isolates susceptible to autologous neutralization (11/16 isolates, 68.7%), when positive autologous neutralization was defined as the capacity to neutralize more than 50% of the virus in comparison to viral detection in control wells in absence of antisera. Preliminary data indicate that this percentage is probably higher than would be detected in a greater sample (15/26 isolates, 57.7%). When 80% activity reduction is defined as positive neutralization, 7/16 (43.7%) of the isolates tested were neutralized by their autologous antisera. Athough no sistematic titration of NAb was carried out, the great majority of the autologous plasma neutralized the autologous HIV-1 isolate at very low titers (1:10 - 1:20). Only four (15.4%) of the isolates appear to be highly susceptible to the autologous neutralizing antibodies, as detected by the capacity of autologous NAb to neutralize higher viral concentrations. Only one (6.7%) of the primary isolates was completely neutralized by the autologous NAb (two separate assays).

The majority of the samples with positive autologous NAb were detected in individuals with a seroconversion period of less than one year and with higher CD4 positive T cell counts, as reported in other studies (M Arendrup et al. 1992 *J AIDS 5:* 305-307, ML Tsiang et al. 1994 *J Inf Dis 170:* 1141-1147), although no statistically significant difference between groups of patients distributed according to infection time and CD4 T cell count was found. No correlation to stage of disease, sex, age or probable mechanism of infection could be detected.

Heterologous neutralization of primary HIV-1 isolates from Rio de Janeiro was highly variable, as reported in studies from other geographical areas (WHO Network for HIV Isolation and Characterization Report). Fifty three out of 75 assays (70.7%) indicated a greater than 50% heterologous neutralization. Some HIV-1 primary isolates showed a higher susceptibility (7/21, 33%) to neutralization, being neutralized by the majority of heterologous plasma tested (varying from 2/3 to 9/9 assays) others were quite resistant to heterologous neutralization (2/21, 9.5%), being neutralized by only one of the heterologous antisera (1/3 to 1/6).

An attempt to analyse difference in susceptibility to neutralization by plasma from individuals infected with the Brazilian **B'** HIV-1 subtype variant or the more international **B** variant was carried out but results showed a qualitative difference for only one isolate. Quantitative studies may allow distinction.

Susceptibility to pools of plasma from seropositive individuals was higher when pools of plasma of asymptomatic individuals were employed (12/12, 100%) than when mixtures of plasma from individuals in more advanced stages of the disease were used (17/20, 85%). Susceptibility to specific anti-HIV-1 MN serum was higher than expected (6/12, 50%).

Neutralization (>50%) of the reference HIV-1 isolate MN was achieved by 25/28 (89%) of the plasma, confirming results reported in the literature (V Bongertz et al. 1994 *Mem Inst Oswaldo Cruz 89:* 113-114). Although the capacity to neutralize the HIV-1 MN isolate does not correlate to the capacity to neutralize either the autologous or heterologous primary isolates, the reverse situation may indicate a special lack of capacity to neutralize either autologous or heterologous isolates: of the three plasma unable to neutralize the HIV-1 MN isolate, none did neutralize the autologous isolate, and capacity to neutralize heterologous primary HIV-1 isolates was quite low.

The neutralization assay described recently (S Zolla-Pazner 1994 *9éme Coll Cent Gardes* 161-165) as a more sensitive test for HIV-1 neutralization was also used for determination of neutralization susceptibility of Brazilian primary HIV-1 isolates. In this assay, the normal human peripheral blood lymphocytes (PBMC) are not pre-activated by incubation with PHA, i.e., "resting PBMC" are employed as host cells for HIV-1 infection. Our results indicate that although the tissue culture infective dose 50% (TCID 50%) of the reference HIV-1 MN isolate used in our assays was the same in both kinds of assays (1:62.500), the TCID 50% of the Brazilian primary isolates showed a reduction (between 2 and 20) of their TCID 50%, pre-

venting general employment of this assay for evaluation of our isolates and antisera.

Attempts to correlate clinical data or other data available (reactivity with synthetic peptides, genotyping results) referent to the primary isolates/plasma analyzed showed no significant results. It should be noted, however, that although neither of the 2 F subtype HIV-1 primary isolates were neutralized by their autologous antisera, the neutralization of B subtype primary isolates by sera from individuals infected with the F type isolates was very high, as well as the neutralization of the F subtype isolates by sera from individuals infected with B subtype HIV-1 isolates.

When potency of heterologous neutralization (50%) was compared to susceptibility to heterologous neutralization (50%) of the primary isolates, an inverse relationship appeared to exist in that plasma from individuals infected with an isolate with greater susceptibility to heterologous neutralization showed a generally poorer capacity to neutralize other primary HIV-1 isolates. Experiments envolving a larger sample number to allow statistical analysis are being carried out.

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